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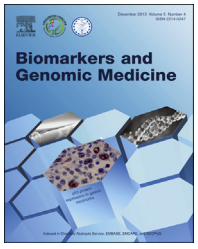

REVIEW ARTICLE

Hypoxia-induced signaling and its relevance in discovering biomarkers for cancer research

Sergio Manzetti [a,b,*], **Oergis Tzamneti** [a]

[a] *Fjordforsk Institute for Science and Technology, Fresvik 6896, Norway*
[b] *Computational Systems and Biology, Department of Cell and Molecular Biology, Biomedical Centre, University of Uppsala, Uppsala, Sweden*

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Abstract Hypoxia is a condition of progressive depletion of cellular oxygen, encompassing a variety of factors that have a sensitive interplay with the nucleus, DNA, and protein machinery. The relevance of hypoxia with cancer biology has been increasingly observed in recent years; however, currently there are major clinical obstacles in understanding the mechanism of tumor progression and identifying the correct therapy. This review sheds light on the most recent findings on hypoxia-induced factors that are involved in cancer progression, and relates them to a network of signals that are co-involved in tumor growth. In this perspective, this review elaborates on unanswered key questions with regard to regulation and modulation pathways related to oxygen-deprived conditions during cancer development, including a brief view of specific microRNA factors in hypoxia. The focus of this review is on the vast landscape of components that are involved in tumor progression, including identification of potential targets and pathways that can play a pivotal role in identifying clinical and diagnostic methods, with hypoxia as a starting point. Defining novel and potential cell cycle factors is of significant importance, particularly given the increasing emergence of personalized medicine.

* Corresponding author. van der Spoel Lab, Computational Systems and Biology, Department of Cell and Molecular Biology, Biomedical Centre, University of Uppsala, Sweden Box 256, 751 05 Uppsala, Sweden.
E-mail address: sergio.manzetti@fjordforsk.no (S. Manzetti).

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Introduction

Hypoxia is a condition of progressive depletion of oxygen caused by poor circulation to the cell or tissue. Oxygen depletion principally creates a hostile condition to all kinds of viable cells but this phenomenon regularly occurs when the tumor volume exceeds a certain limit and thus serves as an important contributor for the growth of cancer cells, such as in cases of ovarian, breast, and hepatocellular carcinomas.[1–3] The process of cancer cell growth and its progression includes several steps and events at the biochemical level and depends on a vast number of cellular and extracellular factors that are altered during the transformation from a normal cellular condition to a cancer cell. Early stages of cancer are often triggered and fueled by hypoxia, regulatory imbalances in T-cell expression, aberrant homeostasis, neoangiogenesis, endocrine, neurocrine, paracrine and autocrine triggering, and hormonal imbalances.[2,4–17] Hypoxia, in particular, leads to a series of events taking place in the cell, altering its metabolic potential and its growth-regulating mechanisms. Moreover, it constitutes a major clinical obstacle in terms of tumor progression and therapy, which is often diagnosed by biopsies and serological profiling.

During serological and sample analysis, identifying the expression patterns of cell factors are of pivotal importance, as they have been for the past 70 years in diagnostic medicine. For this formidable task, biomarkers have been developed for diagnostic purposes based on their involvement in cell cycles, tumor progression, angiogenic responses, tissue remodeling, and other pathophysiological processes.[16,18–22] This particular approach of biomarking disease evolved after the Second World War, when the first protein-expression patterns and metabolites were studied in the context of heart disease and neuronal cancer.[23,24] Since then the vast majority of diseases, particularly hereditary, have been directly linked to a deficiency in expression patterns of pivotal proteins and enzymes, with the most recognized example being the p53 tumor suppressor.[25,26] Diagnostic and prognostic importance of a cancer biomarker concept is thus derived from sound and accurate tissue analysis with a direct correlation to the patient's clinical parameters, and requires that the candidate biomarker has a central presence in a cancer-associated biochemical pathway.

This review focuses on the basic mechanisms of hypoxia-related signaling pathways, which are central responses in cell-survival reflexes. In light of the tumor biological perspective of hypoxia, the key findings on the hypoxia-inducible factor (HIF) family of proteins and their regulatory response to oxygen-deprived conditions are comprehensively summarized and elaborated, particularly with regard to the microRNA (miRNA) network, which is an emerging and increasingly studied field in tumor biology. Also, the review describes how the knowledge about hypoxia-related pathways can aid in identifying novel molecular biomarkers for clinical utility.

HIF proteins and oxygen-dependent regulation

In oxygen-deprived conditions, the most crucial event that takes place in the cell is the induction of the HIF proteins. The HIF proteins regulate and effectuate the transcription of numerous hypoxia-inducible genes during oxygen depletion to increase cell-survival potential and constitute a direct reference to tumor progression and aggressiveness.[14,27] The HIF transcription factor 1 is a heterodimeric protein of the basic helix–loop–helix PAS family of DNA-binding proteins found in all metazoans, and uses its heterodimeric structure to induce the expression of hypoxia-responsive genes.[28,29] HIF-1α and HIF-2α contain an oxygen-dependent degradation domain (ODDD),[30,31] making their expression highly oxygen dependent. By contrast, the HIF-1β unit, also referred to as aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed.[32] Under normoxic conditions, the von Hippel–Lindau (VHL) tumor suppressor binds to the ODDD and facilitates the degradation of the HIF proteins by the 26S proteasome system.[33] The recognition of HIF-1α subunits by VHL is in turn mediated by the activity of prolyl hydroxylase proteins, which confer enzymatic hydroxylation of specific prolyl residues within the HIF ODDD in an oxygen-dependent manner.[34,35] The survival responses induced by HIF are shear responses to an oxygen- and nutrient-deprived state of the cell, and the survival mechanism triggers a cell cycle, which has features similar to tumorigenesis (e.g., growth and proliferation).

During their survival-inducing function, the HIF proteins seem to be utterly regulated by oxygen concentrations; however, increasing evidence suggests that the accumulating HIF proteins also exhibit relevant functions beyond oxygen dependence.[36] Saito and colleagues[36] demonstrated that HIF-2α was induced by the RELA protein, independently of oxygen-dependent hydroxylation. The RELA protein is a nuclear factor of the κB family, and constitutes an essential function for endochondral ossification in cultured chondrocytes and embryonic skeletal growth in mice.[37] Moreover, the oxygen-independent HIF-1α/ARNT activity during normoxic conditions in keratinocytes involves a yet uncharacterized oxygen-independent activity of the HIF in response to normal physiological conditions of the epidermis.[38] These recent findings jointly suggest that HIF proteins are exerting regular maintenance functions during normal physiological conditions, but become crucial growth-inducing factors during induced cell shocks, such as oxygen depletion. This very aspect of carrying a strong survival-promoting potential, disguised by regular cell function, is a key aspect in delineating putative biomarkers in the future, and can directly aid in resolving deeper alternative functions of seemingly regular genes in the cell machinery triggered by extreme conditions, such as during hypoxic signaling.

Hypoxic signaling and its implication in cancer

High expression of HIF-1α is pivotal in a variety of malignancies, encompassing brain, lung, esophageal, stomach, colorectal, prostate, uterine, and cervical carcinomas, and the transcription of their downstream genes promotes tumor growth and survival. In particular, it has been shown that HIF-1α and HIF-2α are jointly expressed and corroborate in the context of physiological and pathological hypoxic conditions in rat insulin-secreting pancreatic β cells[39] or in the early stages of esophageal cancer.[40] One

of the functional explanations for elevated HIF-1α levels in cancer is supposed to be the loss of VHL function[29,34]; however, in the following section, we present recent evidence that HIF expression can also be dysregulated and affected by tumor-associated signaling pathways. In addition, we also comment on selected HIF-induced transcriptional targets and HIF's ambivalent contribution to a cancer phenotype in terms of oncogenic and tumor-suppressive settings.

HIF regulation in cancer

The molecular framework for altered HIF expression in cancer was recently complemented by Pore et al.[41] It was demonstrated that the phosphoinositide 3-kinase (PI3K) signaling pathway—mediated by Akt1—induces HIF-1α expression. However, given the finding that inhibition of mammalian target of rapamycin with rapamycin had no significant effect on HIF-1α expression. Pore and et al[41] claimed that the contribution of an additional and so far unknown mediator downstream of AKT could control HIF expression.[41] Moreover, Brugarolas and co-workers[42] showed that loss of TSC2, an important downstream component of the PI3K axis, results in HIF-1α accumulation, which in turn stimulates transcriptional activation of vascular endothelial growth factor (VEGF), an important downstream target of HIF-1α.

Complementarily to these findings, Cheng and co-workers[43] reported on an interesting observation with regard to PI3K/AKT downstream effects. They demonstrated that Egr-1 is involved in Epidermal growth factor (EGF)-induced growth and invasiveness of ovarian cell lines. This effect depends on the PI3K/AKT signaling pathways and involves Egr-1 as the downstream component. Using the small interfering RNA approach, Cheng and co-workers[43] showed that Egr-1 expression downregulated E-cadherin by inducing its transcriptional repressor Slug. Thus, the potential participation of Egr-1/EGF in the PI3K/AKT pathway, which is also highly relevant in a hypoxic condition and contributes to HIF-1α upregulation, might suggest a potential link to the "missing pieces" in the puzzle of HIF-1α regulation, as quoted by Pore et al.[44] Given the fact that the EGF axis is also highly relevant for tumorigenesis, a possible interplay between EGF signaling and hypoxia-related pathways can be hypothesized.

The cytokine 15-deoxy-delta12,14-prostaglandin J_2 (PGJ_2) is supposed to be a key endogenous negative regulator of inflammation and angiogenesis,[45] which can be used by cells to resolve an inflammatory response.[46–48] In this context, Zimmer et al showed that PGJ_2 translationally inhibits HIF-2α by recruiting iron regulatory protein-1 (IRP1), which is involved in regulating the transcriptome network and is dependent on an iron-responsive element within the 5′-untranslated region of the HIF-2α message.[49,50] Interestingly, this was supposed to be the first report showing that anti-inflammatory and putative antineoplastic effects of PGJ_2 may be mediated through inhibition of HIF-2α within the tumor epithelial cells and/or mesenchymal cells of the tumor microenvironment. Given that HIF-2α is expressed during hypoxia and tumor-promoting inflammatory cytokines are triggered,[51] it is feasible that the IRP1 might be downregulated by an unknown oncogenic factor, which in turn induces HIF-2α to produce protumorigenic effects. Conspicuously, the study by Fang and co-workers[51] is of particular interest, as it shows a connection between HIF-2α, inflammation, and cancer. Thus, a cellular response that should normally resolve an inflammatory condition might be "misapplied" by cancer cells to increase angiogenic capacity, thus promoting tumorigenesis through iterative inflammation.

Recent findings suggest that estrogen receptor (ER) signaling can directly interfere with HIF transcriptional activity in a tumor biological context. It was demonstrated that ER-β contributes to ARNT downregulation.[52] Thus, a potential tumor-suppressing function of the estrogen-β receptor takes place by attenuating the induction of VEGF, an important HIF-1α downstream target (Fig. 1). By decreasing the formation of functionally active HIF-1α/ARNT complexes and depleting VEGF levels, ER-β might impair the tumor's angiogenic response,[52] which makes estrogen a potential target of repression by hypoxia-mediated tumorigenic responses.

HIF's transcriptional targets and their ambivalent roles

In macrophages experiencing hypoxia, HIF-1α and HIF-2α regulate transcription of a variety of cytokines and chemokines, including vasodilating adrenomedullin, VEGF A, interleukin-1β (IL-1β) and IL-8, CXCR4, and angiopoietin-2.[39,51] Of the many potent tumor-promoting cytokines, adrenomedullin is a protein that facilitates angiogenesis through paracrine and autocrine pathways and it was recently found to be involved in the progression of melanoma.[53]

Wang and et al[54] recently suggested that HIF-1α transcriptional targets can have a direct impact on oncogenic Epidermal growth factor receptor (EGFR) signaling. It was demonstrated that calveolin-1 expression is induced by HIF-1α, which accentuates the formation of specialized raft lipid microdomains (caveolae). This in turn promotes EGFR dimerization followed by its ligand-independent activation.[54] These findings are of particular interest as they underscore a further mechanism by which HIF transcriptional targets contribute to the oncogenic potential of tumor cells. It is shown that tumor cells can bypass the dependence on EGF ligand and adapt downstream signaling under hypoxic conditions.

The aforementioned studies implied a rather tumor-promoting role of HIF; however, there are contradicting findings on the function of HIF proteins. Wild-type HIF-1α was recently found to act as a tumor suppressor rather than an oncogene in kidney cancers harboring 14q deletions.[55] The findings by Shen et al[55] suggest that HIF-1α requires pivotal cofactors expressed at the 14q locus to carry out its tumor-promoting activity during hypoxia, but these are largely unknown. The requirement of these unknown factors may also be partially linked to a recent study on a mouse model for inflammatory kidney disease by Kobayashi et al.[56] Kobayashi et al[56] showed that HIF-1α suppresses inflammation and fibrogenesis by directly or indirectly downregulating CC chemokine receptors in

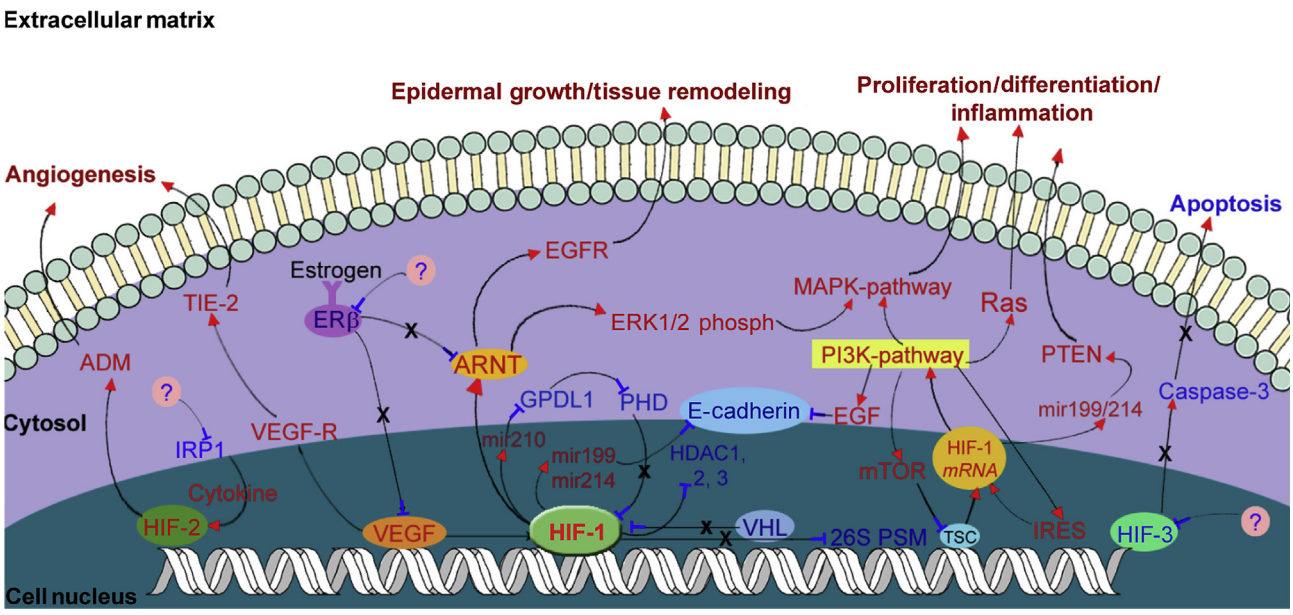

Figure 1 Hypoxia-induced oncogenic pathways. The complex network of HIF-inducible factors is depicted in relation to the most defining cellular processes that sustain oncogenesis, angiogenesis, proliferation, differentiation, inflammation, remodeling, and growth. Red arrows indicate stimulation, whereas blue arrows indicate suppression. The same color pattern is arranged for the names of cellular and nuclear factors. HIF-1, HIF-2, and HIF-3 denote HIF-1α, HIF-2α, and HIF-3α4, respectively. HIF = hypoxia-inducible factor; ARNT = aryl hydrocarbon receptor nuclear translocator; ADM = adrenomedullin; ER = estrogen receptor; EGFR = epidermal growth factor receptor; ERK = extracellular signal-regulated kinase; GPDL1 = glycerol-3-phosphate dehydrogenase 1-like protein; IRP = iron-regulatory protein; MAPK = mitogen-activated protein kinase; mRNA = messenger RNA; mTOR = mammalian target of rapamycin; PTEN = phosphatase and tensin homolog; VEGF-R = vascular endothelial growth factor receptor.

macrophages. Considering that inflammation is a cellular reaction that can mediate cancer,[57] the role of HIF-1α in suppressing inflammation shows that there are presently missing pieces in our understanding of hypoxia-induced cancer network. The question on which factors or context is needed that make HIF proteins act in a pro- versus antitumorigenic manner may thus be a crucial avenue to be investigated in future cellular studies and may serve to complete our picture on how hypoxia can "fuel" cancer.

HIF-3α: A suppressor of HIF-associated tumorigenesis?

HIF-1α and HIF-2α subunits have two transactivation domains, an N-terminal transactivation domain (NAD) and a C-terminal transactivation domain, which are involved in DNA binding and transcriptional regulation.[58,59] The HIF-3α subunit differs from HIF-1 and HIF-2α and contains only a NAD.[60] So far, only a little is known about the expression pattern and function of HIF-3α in human cells. A recent investigation verified HIF-3α expression in the human kidney and showed that this subunit suppresses HIF-mediated gene expression.[60] In this context, the authors hypothesized a competition of HIF-3α and HIF-1α or HIF-2α for the recruitment of ARNT factor. Complementarily, HIF-3α4, an alternatively spliced variant of human HIF-3α, was shown to form a complex with HIF-1α and HIF-2α, which has an abortive role on the transactivation of hypoxia-response elements in terms of negative regulating factor. Moreover, HIF-3α4 was shown to be dramatically downregulated in primary renal carcinomas and confers a growth-suppressive effect in VHL-null renal carcinoma xenografts by decreasing HIF-2α-mediated gene expression and has been suggested as a putative tumor suppressor.[61,62] In accordance with these findings, Torii and co-workers[63] investigated the inhibitory PAS (Per/ARNT/Sim) domain protein (IPAS), a further alternatively spliced variant of HIF-3α, and described it in the context of a dominant negative feedback inhibitor of HIF-dependent hypoxic response. Interestingly, besides the known contribution of HIF-3α splice variants to transcriptional repression, the authors sophisticatedly demonstrated that IPAS critically interferes with the interaction of Bcl/x_L with Bax and induces apoptosis in prostate carcinoma cells.[63]

Hypoxia-induced signaling and the identification of novel biomarkers

Detailed knowledge about hypoxia-associated signaling pathways is useful to identify novel cancer biomarkers. In a recent investigation by Tong and co-workers,[64] HIF-1α expression in the bone marrow was determined by immunohistochemical analysis. The group predicted poor survival and recurrence of myelodysplastic syndrome, revealing a central clinical role for the HIF axis in pathology. In their study, Tong and co-workers[64] analyzed samples from patients with surgically treated intracranial meningiomas for

hypoxia- and angiogenesis-related factors. Parallel to this study, the expression of HIF proteins and VEGF was found to be a good predictor of tumor recurrence[65] as well as a good biomarker for early stages of esophageal cancer along with the BCL-2 expression, which is highly oxygen dependent.[40] Thus, based on the aforementioned studies, not only HIF but also the downstream components in this axis might be promising biomarkers in tumor pathology.

One of these downstream components is related to the expression of the voltage-dependent anion channel 1 (VDAC1-ΔC), which is interestingly expressed in patients with lung cancer.[66]

In addition, for other neurological malignancies such as glioblastoma, hypoxia has been described as a challenging clinical obstacle. Glioblastoma has a high metabolic demand, leading to relative tissue hypoxia and necrosis, which confers therapy resistance.[67] Monitoring tissue hypoxia in those patients and estimating therapy response are of significant clinical interest. Therefore, Tateishi and coworkers[68] recently described a method of noninvasive oxygen measurement in the brain by hypoxic positron emission tomography imaging. In this context, they used ^{62}Cu-ATSM as a radiotracer. Uptake of this tracer into the cells is dependent on cellular oxygen concentration. ^{62}Cu-ATSM uptake was shown to predict HIF-1 positivity in the tumor tissue, providing an auxiliary diagnostic tool to evaluate tumor hypoxia in patients with glioblastoma. Not only single components of hypoxia-induced signaling pathways, but also global signatures of hypoxia-inducible genes were shown to be predictive for prognosis and therapy efficiency[69,70] in cancer patients.

Conclusion

The mechanism of hypoxia depends on a variety of cellular factors, with HIF proteins playing a central role. Recent findings enlarge our picture of HIF in cancer and imply a complex interplay with cytokine networks and important signaling pathways including EGFR, PI3K, or ER axes (Fig. 1). In this context, the cancer cell's adaption to a hypoxic microenvironment seems to be an essential "fueling" mechanism for tumor progression. Upon metabolic adaption to an oxygen-deprived condition, cancer cells become more aggressive or even completely therapy resistant. However, evidence is accumulating that hypoxic signaling does not exclusively contribute to oncogenic effects and could also be associated with tumor-suppressive and proapoptotic cascades.

Presently, understanding and interpretation of hypoxic-response biology is highly fragmented. There are a lot of "missing parts" in the molecular puzzle of HIF and its downstream effects, and further efforts will have to be carried out to identify factors that are necessary for distinguishing oncogenic from tumor-suppressive and proapoptotic responses of hypoxia-induced signaling. In addition, the miRNA network, which is briefly discussed here, seems to comprise fundamental mediators of hypoxic response and hypoxia-related tumorigenicity. Considering this involvement of miRNA networks, our picture of hypoxic-response biology is complicated even further; however, specific and sensitive assays for miRNA detection as well as molecular tools for miRNA-expression modulation are available.

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